

## ***Ascotricha microspora* sp. nov. from Cayman Islands**

DE-WEI LI<sup>1, 2\*</sup> & GUIHUA ZHAO<sup>3</sup>

<sup>1</sup> The Connecticut Agricultural Experiment Station, Valley Laboratory,  
153 Cook Hill Road, Windsor, CT 06095

<sup>2</sup> Southern China Collaborative Innovation Center of Sustainable Forestry,  
Nanjing Forestry University, Nanjing, Jiangsu, 210037 China

<sup>3</sup> Center of Biotechnology R&D, Jiangsu Polytechnic College of Agriculture & Forestry,  
3 Changjiang Road, Jurong, Jiangsu 212400, China

\* CORRESPONDENCE TO: [dewei.li@ct.gov](mailto:dewei.li@ct.gov)

**ABSTRACT**—A new hyphomycete species, *Ascotricha microspora* collected from Cayman Islands in the Caribbean, is described and illustrated. The asexual fungus differs from *Dicyma vesiculifera* by having smaller, colorless conidia. *Ascotricha rugispora* (= *Surculiseria rugispora*) and *A. funiculosa* (= *Dicyma funiculosa*) are proposed as new combinations.

**KEY WORDS**—ITS, microfungi, phylogeny, saprobe, Xylariaceae

## **Introduction**

*Ascotricha* Berk. was proposed as a monotypic ascomycete genus (Berkeley 1838) typified by *A. chartarum* Berk. Boulanger (1897) typified *Dicyma* Boulanger by *Dicyma ampullifera* Boulanger, which he described as the conidial state of *Chaetomium zopfii* Boulanger [= *Ascotricha zopfii* (Boulanger) Peyronel], all names that were later synonymized with *Ascotricha chartarum* (Hawksworth 1971). Von Arx (1982) concluded that *Dicyma* is synonymous with *Hansfordia* S. Hughes, *Basifimbria* Subram. & Lodha, *Gonytrichella*, and *Puciola* De Bert. Nonetheless, some mycologists still regard *Hansfordia* and *Basifimbria* as distinct genera (Seifert & al. 2011). As the type species of *Dicyma* 1897 (*D. ampullifera*) and *Ascotricha* 1838 (*A. chartarum*) are

TABLE 1. ITS sequences of *Ascotricha microspora* and allied taxa used for phylogenetic analysis.

| TAXON                         | ACCESSION NO.*                                   | SUBSTRATE                          | LOCALITY         | ITS      | REFERENCE              |
|-------------------------------|--------------------------------------------------|------------------------------------|------------------|----------|------------------------|
| <i>Ascotricha bosei</i>       | CBS448.93 [T]                                    | Date palm plantation soil          | Iraq             | KF893283 | Cheng & al. 2015       |
| <i>Ascotricha chartarum</i>   | CBS234.97                                        | Tropical rain forest soil          | Papua New Guinea | KF893284 | Cheng & al. 2015       |
|                               | B4C                                              | Oil on canvas painting             | Nigeria          | KX246915 | Okpalanozie & al. 2016 |
|                               | B4F                                              | Oil on canvas painting             | Nigeria          | KX246917 | Okpalanozie & al. 2016 |
| <i>Ascotricha erinacea</i>    | S98                                              | <i>Dendrobium officinale</i>       | China            | KT224876 | —                      |
|                               | CBS535.73                                        | Elephant dung                      | East Africa      | KF893285 | Cheng & al. 2015       |
|                               | CBS407.87                                        | Paper and cardboard                | Netherlands      | KF893287 | Cheng & al. 2015       |
|                               | OUCMBIII <sub>10</sub> 1069                      | Sediment                           | China            | KF893281 | Cheng & al. 2015       |
|                               | NBRC31999                                        | Leaf of broad-leaved tree          | Israel           | 03199901 | —                      |
|                               | NBRC9841                                         | Urethane foam                      | —                | 00984101 | —                      |
|                               | NBRC33232                                        | House dust                         | Japan            | 03323201 | —                      |
| <i>Ascotricha guamensis</i>   | NBRC9991 (IMI 82619)                             | Soil                               | —                | 999103   | —                      |
| <i>Ascotricha longipila</i>   | CGMCC 3.15229<br>(OUCMB <sub>18</sub> ) [T]      | Marine algae                       | China            | KC503896 | Cheng & al. 2015       |
| <i>Ascotricha lusitanica</i>  | S19                                              | <i>Dendrobium officinale</i>       | China            | KT224797 | —                      |
|                               | CBS462.70 [IT]                                   | <i>Cicer arietinum</i> seed        | Portugal         | KF893289 | Cheng & al. 2015       |
| <i>Ascotricha microspora</i>  | NHES L1706 [T]                                   | Unidentified decaying leaf         | Cayman Islands   | MF805818 | This study             |
| <i>Ascotricha parvispora</i>  | CGMCC 3.14995<br>(OUCMBI <sub>11</sub> 0001) [T] | Living <i>Enteromorpha linza</i>   | China            | JX014298 | Cheng & al. 2015       |
| <i>Ascotricha sinuosae</i>    | CGMCC3.149965<br>(OUCMBI <sub>11</sub> 1190) [T] | Living <i>Colpomenia sinuosa</i>   | China            | JX014299 | Cheng & al. 2015       |
|                               | A1S5-10                                          | Beach soil                         | Malaysia         | KJ780756 | —                      |
|                               | F8                                               | Plant endophyte                    | Sudan            | KR673905 | —                      |
| <i>Daldinia bambusicola</i>   | E40F                                             | Agarwood tree                      | India            | KY425743 | —                      |
| <i>Daldinia childiae</i>      | 2-00219-2                                        | <i>Platycladus orientalis</i> leaf | China            | KT192354 | —                      |
| <i>Daldinia eschscholtzii</i> | JMRC SF 11930                                    | <i>Paphiopedilum exul</i> root     | Thailand         | KU304335 | Barnes & al. 2016      |

|                                |                    |                                                                        |             |          |                            |
|--------------------------------|--------------------|------------------------------------------------------------------------|-------------|----------|----------------------------|
| <i>Daldinia petriniae</i>      | H Knudsen s.n. (C) | <i>Corylus avellana</i>                                                | Denmark     | AF176970 | Johannesson & al. 2000     |
| <i>Dicyma funiculosa</i>       | CBS124.80 [T]      | Soil                                                                   | Spain       | KF893286 | Cheng & al. 2015           |
|                                | CBS323.86          | Forest soil                                                            | Spain       | AY908992 | U'Ren & al. 2016           |
| <i>Dicyma pulvinata</i>        | 414-3              | —                                                                      | —           | LC177968 | Unpublished                |
|                                | KACC44502          | —                                                                      | —           | LC177971 | Unpublished                |
|                                | CBS194.56          | <i>Prunus persica</i> twig                                             | Italy       | AY908993 | U'Ren & al. 2016           |
|                                | LCC11              | Giant panda vaginal secretions                                         | China       | KF986550 | —                          |
|                                | 5023               | <i>Eucalyptus globulus</i> leaves                                      | Spain       | FR667996 | Sánchez Márquez & al. 2011 |
| <i>Hansfordia nebularis</i>    | CBS254.59          | <i>Lycopersicon esculentum</i> leaf                                    | Netherlands | AY908995 | Peláez & al. 2008          |
| <i>Hansfordia pulvinata</i>    | CBS115626          | <i>Quercus robur</i>                                                   | Italy       | KF893290 | Cheng & al. 2015           |
|                                | HMQAU130012        | Tomato leaf?                                                           | China       | KP772241 | —                          |
|                                | KACC44498          | Hyperparasitizing<br><i>Pasadora fulva</i> on<br>organic tomato plants | Korea       | HM060587 | —                          |
|                                | NBRC30211          | <i>Lithocarpus edulis</i>                                              | Japan       | 03021101 | —                          |
|                                | NBRC6791           | —                                                                      | —           | 00679101 | —                          |
|                                | NBRC9007           | —                                                                      | —           | 00900701 | —                          |
| <i>Lopadostoma turgidum</i>    | CBS 133207 [T]     | <i>Fagus sylvatica</i>                                                 | Austria     | NR132036 | Jaklitsch & al. 2014       |
| <i>Podosordaria muli</i>       | WSP167 [T]         | Mule dung                                                              | Mexico      | GU324761 | Hsieh & al. 2010           |
| <i>Surculiseries rugispora</i> | NBRC33167 [T]      | <i>Bruguiera gymnorhiza</i> leaf                                       | Japan       | LC146763 | Okane & al. 2001           |
|                                | NBRC33168          | <i>Bruguiera gymnorhiza</i> leaf                                       | Japan       | 03316801 | Okane & al. 2001           |
| <i>Xylaria hypoxylon</i>       | ATCC42768 [T]      | Wood                                                                   | USA         | AY327477 | Platas & al. 2004          |

\* [T] = ex-holotype; [IT] = ex-isotype.  
ATCC = American Type Culture Collection, Manassas, United States; BICC = Biocon culture collection, India; BCC = BIOTEC Culture Collection, Thailand; CBS = Westerdijk Fungal Biodiversity Institute (formerly Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; HMQAU = Fungal Herbarium of Qingdao Agricultural University, Qingdao, China; IMI = IMI Fungarium, the Royal Botanic Gardens, Kew, UK; KACC = Korean Agricultural Culture Collection, South Korea; MFLUGC = Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; NBRC = the NITE Biological Resource Center, Japan; NFCCI = the National Fungal Culture Collection of India; NRRL = The Agricultural Research Service Culture Collection, USDA, USA; OUCMB = Herbarium of Ocean University of China, Marine Biology, Shandong, China; UAMH = UAMH Centre for Global Microfungal Biodiversity, University of Toronto, Canada; WSP = Washington State University, Pullman, Washington, U.S.A.

synonyms (Hawksworth 1971), the generic names are likewise synonymous, with *Ascotricha* having priority as the earlier name.

Here we describe a new asexual species in *Ascotricha*, collected from Cayman Islands.

### Materials & methods

The fungus was collected from fallen leaves and isolated on malt extract agar and incubated at 25°C. Conidiophores, conidiogenous cells and conidia were mounted in 85% lactic acid. A staining agent, 0.1% lacto-fuchsin, was used to observe conidiogenous cells and septation of colorless conidia. All microscopic observations were made under Nomarski differential interference contrast optics of Olympus BX40 microscope. Photomicrographs were taken with an Olympus Microfire digital camera. Measurements of the fungal structures were statistically analyzed with Microsoft Office Excel 2013 with 95% confidence interval of means. The results are presented as ranges calculated based on mean  $\pm$  standard deviation with  $n$  = number of fungal structures measured. The holotype specimen is conserved in the Herbarium, Connecticut Agricultural Experiment Station, New Haven, U.S.A. (NHES).

DNA extraction and ITS sequencing procedures followed Li & al. (2008) as carried out by Gen Script (Nanjing) Co., Ltd. (Nanjing, China). The ITS sequence underwent BLAST analysis via the NCBI web site ([www.ncbi.nlm.nih.gov/blast/](http://www.ncbi.nlm.nih.gov/blast/)) for sequence similarity searches and comparisons. Forty-six sequences representing *Ascotricha*, *Dicyma*, *Hansfordia*, and several genera of *Xylariaceae* and allied families were chosen for phylogenetic analysis (TABLE 1). Sequences are aligned with Guidance2 (mafft) (<http://guidance.tau.ac.il/ver2/>). *Lopatostoma tigidum* CBS 133207 was included as outgroup. The ITS sequence has been deposited in GenBank. Phylogenetic analysis was conducted using MEGA7 (Kumar & al. 2016). Bootstrap test was carried out with 1000 replicates.

### Results

Our phylogenetic analysis distinguished *A. microspora* from other *Ascotricha*/*Dicyma* species and supported it as sister to *Surculiseria rugispora*. As can be seen in FIG. 1, *Ascotricha microspora*, *A. bosei*, *A. chartarum*, *A. erinacea* [= *Dicyma olivacea*], *A. guamensis*, *A. longipila*, *A. lusitanica*, *A. parvispora*, *A. sinuosae* [ $\equiv$  *Hansfordia sinuosae*], *Dicyma funiculosa*, and *Surculiseria rugispora* group within the same clade (the /*Ascotricha* clade) with 97% support; the inclusion of the ex-type sequence of the monotypic genus *Surculiseria* within /*Ascotricha* suggests that it is congeneric with *Ascotricha*. FIG. 1 shows all *Dicyma funiculosa* sequences as monophyletic with 99% support and all sequences of *Ascotricha erinacea* [= *Dicyma olivacea*] as monophyletic with 98% support. However, the *Ascotricha chartarum* sequences are not monophyletic, with two sequences (B4C and B4F) grouping

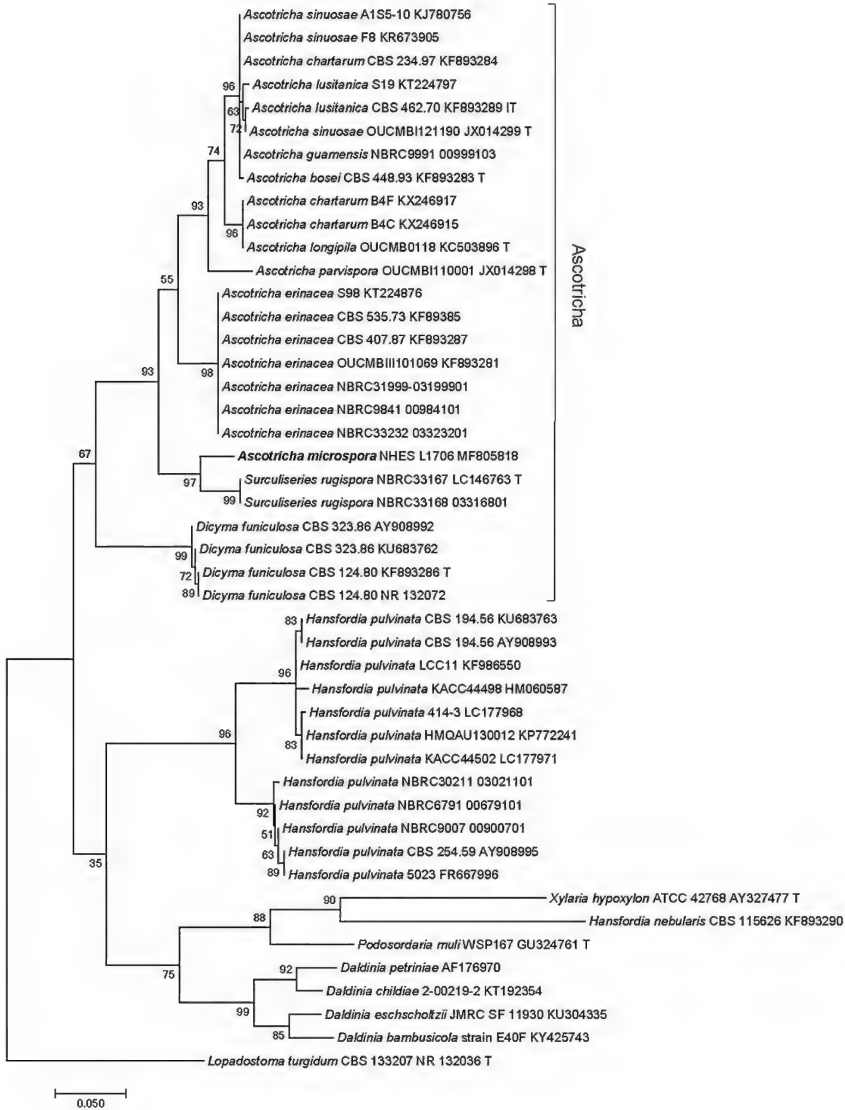


FIG. 1. Maximum Likelihood analysis of *Ascotricha microspora* and allied taxa, based on 46 sequences. *Lopadostoma turgidum* CBS 133207 is included as outgroup. The bootstrap test was conducted with 1000 replicates; bootstrap values >50% are indicated at the nodes. The scale bar indicates the number of expected changes per site. Annotations T and IT indicate the ex-type sequences included in the analysis.

with *A. longipila* while the third (CBS234.97) groups with *Ascotricha bosei*, *A. guamensis*, *A. lusitanica*, and *A. sinuosae* [ $\equiv$  *H. sinuosae*].

All 12 isolates of *Hansfordia pulvinata* [ $\equiv$  *Dicyma pulvinata*] were monophyletic with 96% support and sister to a clade (containing sequences of *Daldinia* and other genera) within which *H. nebularis* grouped with *Xylaria hypoxylon* (FIG. 1). The clear separation between these clades and the /*Ascotricha* clade indicates that *H. pulvinata* and *H. nebularis* do not belong in *Ascotricha*.

## Taxonomy

***Ascotricha* Berk.**, Annals of Natural History 1: 257 (1838)

= *Dicyma* Boulanger, Revue Générale de Botanique 9: 18 (1897)

= *Gonytrichella* Emoto & Tubaki, Transactions of the  
Mycological Society of Japan 11: 95 (1970)

= *Puciola* De Bert., Mycotaxon 3(3): 553 (1976)

= *Surculiseries* Okane, Nakagiri & Tad. Ito, Mycoscience 42(1): 116 (2001)

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FIG. 2

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Differs from *Dicyma vesiculifera* by its smaller, colorless conidia.

TYPE: Cayman Islands, Grand Cayman, fallen leaves, 2 January 2012, De-Wei Li  
(Holotype, NHES L1706 (GenBank MF805818).

ETYMOLOGY: epithet means small conidia.

COLONIES hypophyllous, effused, lanose, gray. Mycelium partly superficial, partly immersed in the substrate, composed of colorless to pale brown hyphae, 1–2  $\mu$ m diam. CONIDIOPHORES differentiated, simple, solitary,  $\leq 220$   $\mu$ m long, 2.5–3.2  $\mu$ m diam, smooth, erect or geniculate, alternately branched, brown to pale brown, gradually tapering toward the apex, septate, usually terminating in a capitate or spatulate cell and often developing a similar cell at each geniculation. The capitate or spatulate cells (drumstick cells) are sterile, colorless, smooth, thin-walled, (10.8–)12–15.4(–17.1)  $\times$  (1.3–)1.4–2(–2.4)  $\mu$ m (mean  $\pm$  SD = 13.7  $\pm$  1.6  $\times$  3.8  $\pm$  0.6  $\mu$ m, n = 12), with an apex enlarged into globose or subglobose shaped vesicle, (2.9–)3.2–4.4(–4.9)  $\mu$ m diam. (mean  $\pm$  SD = 3.8  $\pm$  0.6  $\mu$ m, n = 12). Lateral branches arising singly, in pairs or in whorls, penicillate, colorless or pale brown below and colorless above, thin walled, smooth, up to 50  $\mu$ m long, sometimes developing a thin-walled

FIG. 2. *Ascotricha microspora* (holotype, NHES L0706). a. Conidiophore, conidiogenous cells, and conidia; b. Partial conidiophore and a drumstick cell at geniculation (arrowed); c. Conidiogenous cells and conidia; d. conidia. Scale bars: a = 20  $\mu$ m; b–d = 10  $\mu$ m.



ovoid or pyriform, colorless vesicle laterally just below secondary branches,  $(4.6-5.3-7.8(-8.7) \times (2.5-3-5(-6) \mu\text{m})$ , (mean  $\pm$  SD =  $6.6 \pm 1.2 \times 4 \pm 1 \mu\text{m}$ ,  $n = 10$ ). CONIDIOGENOUS CELLS polyblastic, cylindrical, colorless, smooth, thin-walled,  $(5-6-8(-10) \times (1.3-1.4-1.7(-1.9) \mu\text{m})$ , (mean  $\pm$  SD =  $7 \pm 1 \times 1.57 \pm 0.16 \mu\text{m}$ ,  $n = 21$ ), denticulate, enlarged at the apex bearing a cluster of 3–6 denticles,  $0.5-1.4 \times 0.5-0.9 \mu\text{m}$ ; conidial secession schizolytic. CONIDIA solitary, ovoid or ellipsoid, rounded at the apical end, with a papillate basal end, unicellular, smooth, hyaline,  $(3.7-4.2-4.8(-5) \times (2.1-2.4-2.8(-3) \mu\text{m})$ , (mean  $\pm$  SD =  $4.5 \pm 0.3 \times 2.6 \pm 0.2 \mu\text{m}$ ,  $n = 30$ ).

## Discussion

*Ascotricha microspora* is morphologically rather similar to *Dicyma vesiculifera* Piroz. but differs in conidial color and size, with *D. vesiculifera* distinguished by pale yellow-brown and longer (5–7  $\mu\text{m}$ ) conidia; Pyrozynski 1972). Both species produce conidiogenous cells that are enlarged significantly at the apical end and bear denticles. Conidiogenesis in both species is somewhat similar to that found in *Hansfordia*, and their conidiophores develop thin-walled, colorless capitate/spathulate cells that resemble those in *Dicyma*. Thus, *Ascotricha microspora* and *D. vesiculifera* have a morphology intermediate between those in typical *Dicyma* and *Hansfordia* species.

*Surculiseria* was erected and typified with *S. rugispora* based on its polyblastic, backward-curving conidiogenous cells with conspicuous scars on the flattened denticle tops and ITS and LSU sequence analysis (Okane & al. 2001). *Surculiseria* remains monotypic but shares many morphological characters with *Ascotricha* [= *Dicyma*]. However, *S. rugispora* differs from *A. microspora* in conidial morphology and conidiogenous cells. The phylogenetic analysis of Okane & al. (2001) supports *Surculiseria* as paraphyletic to *Ascotricha* and its asexual state, *Dicyma*. However, our ITS-based sequence analysis supports *Ascotricha* and *Surculiseria* as congeneric (FIG. 1). LSU and SSU phylogenies generated from using available GenBank and NBRC sequences confirm the results of the ITS phylogenetic analysis (unpublished data). We therefore propose a new combination of *S. rugispora* in *Ascotricha*:

***Ascotricha rugispora*** (Okane, Nakagiri & Tad. Ito)

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= *Surculiseria rugispora* Okane, Nakagiri & Tad. Ito, Mycoscience 42(1): 121 (2001)



Our phylogenetic analysis (FIG. 1) also supports *Dicyma funiculosa* in the /*Ascotricha* clade. Consequently, we propose a new combination of *Dicyma funiculosa* in *Ascotricha*:

***Ascotricha funiculosa*** (Guarro & Calvo) D.W. Li & G.H. Zhao, **comb. nov.**

MYCOBANK MB 824983

≡ *Dicyma funiculosa* Guarro & Calvo, Nova Hedwigia 37(4): 641 (1983)

The type species of *Hansfordia* is *H. ovalispora* S. Hughes (Hughes 1951), with no available culture or sequences. The phylogenetic relationship between *Dicyma* and *Hansfordia* remains unresolved. That is also the case with *Basifimbria aurea* Subram. & Lodha, the type species of *Basifimbria* (Subramanian & Lodha 1968). Since there are no sequence data of *Basifimbria* available, we cannot establish whether *Basifimbria* is congeneric with *Dicyma* or *Hansfordia*. Further studies are necessary to determine the phylogenetic relationships among the three genera.

*Ascotricha sinuosae*, *A. lusitanica*, *A. guamensis*, and *A. bosei* (together with one sequence labelled as “*A. chartarum*”) share the same clade, with 96% support. Potential synonymies cannot be resolved because of the lack of support within this clade, and the delineation of the three species needs to be further studied with multiple genes.

The current phylogenetic analyses suggest that *Hansfordia pulvinata* and *H. nebularis* may not belong to *Ascotricha* (FIG. 1), and their placement is yet to be determined.

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